Mapping photoisomerization dynamics on three-state model potential energy surface in bacteriorhodopsin using femtosecond stimulated Raman spectroscopy

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Methods

Sample:

Bacteriorhodopsin extracted from *Halobacterium salinarum* L33 cultures was generously provided by Prof. Xin Zhao (East China Normal University). After obtaining the protein, it was concentrated to 3.5 OD/cm (at 570 nm). The sample was stored at 4°C, protected from light, for up to a week. A UV-Vis spectrophotometer was used for spectral scanning (300–900 nm), yielding the visible absorption spectrum of the protein in the dark-adapted state (orange line in Fig. S1a). After 15 minutes of exposure to a white light lamp, a second scan was conducted to obtain the absorption spectrum in the light-adapted state (green line in Fig. S1a).

The sample was exposed to continuous illumination under a white light lamp for 15 minutes to transition to the light-adapted state before conducting ultrafast spectroscopy measurements, the continuous white light illumination was maintained throughout the measurements to keep the sample in this state. The absorption spectra of the sample exhibited minimal changes before and after the experiments (blue line in Fig. S1a).

Ultrafast spectroscopies:

Transient absorption (TA) spectroscopy was collected using a commercial TA spectrometer (HELIOS, Ultrafast Systems, LLC). The laser source was generated from a Ti: Sapphire laser amplifier system (Astrella USP, Coherent, Inc.). The laser had a central wavelength of 800 nm, 35 fs pulse duration, 7 mJ pulse energy and 1 kHz repetition rate. A portion of the 800 nm laser (2 mJ) was directed into an optical parametric amplifier (OperA Solo, Coherent, Inc.) to generate 570 nm pulses for transient absorption. An optical chopper was used to reduce the repetition rate of the excitation light to 500 Hz. Broadband supercontinuum white light (400 nm - 800 nm) was generated by focusing a low-energy laser onto a 2 mm-thick sapphire crystal. The excitation and probe beams were focused into a 2 mm-thick sample cell while ensuring continuous stirring of the sample using a magnetic stirrer. The experimental conditions were maintained at room temperature.

Femtosecond stimulated Raman spectroscopy (FSRS) was collected using a home-built FSRS setup in the laboratory. The 800 nm fundamental beam was split into three

beams for generating the Raman pump, Raman probe, and actinic pump pulses, respectively. The first beam was focused onto a 2 mm-thick sapphire crystal to generate supercontinuum white light for use as the Raman probe. The second beam was directed into an optical parametric amplification (OperA Solo, Coherent, Inc.) system to generate 570 nm femtosecond pulses as the actinic pump. The third beam was directed through a second harmonic bandwidth compressor (SHBC, Coherent, Inc.) to produce 400 nm picosecond pulses, which were then input into a picosecond optical parametric amplification system (TOPAS-400, Coherent, Inc.) to generate picosecond pulses with a central wavelength of 620 nm (with pulse energy of 200 nJ) for use as the Raman pump. All three beams were co-focused onto the sample using an off-axis parabolic mirror, and the Raman probe was collected and directed to a detection system consisting of a spectrometer (IsoPlane, Teledyne Princeton Instruments) and a camera (PIXIS: 100B, Teledyne Princeton Instruments). The instrumental response function of this system was approximately 150 fs. The experiments were conducted at room temperature.

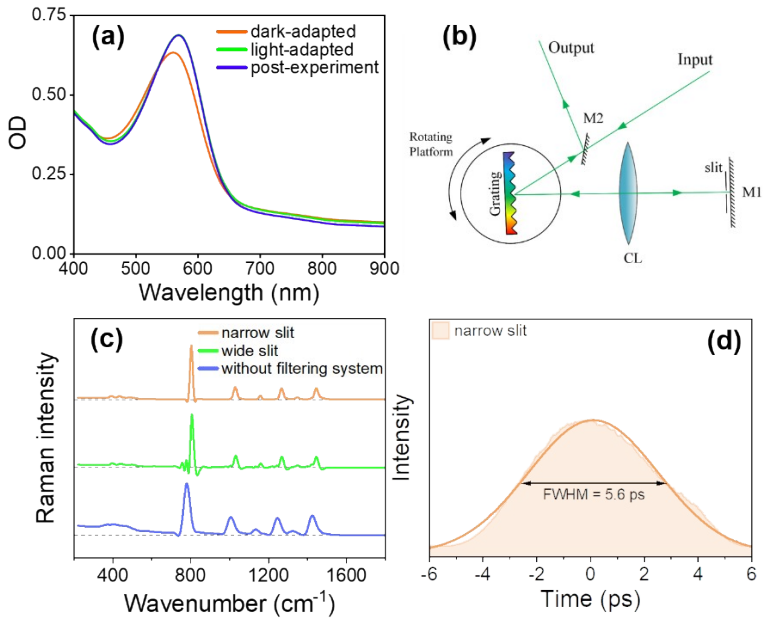

Fig. S1. (a) The absorption spectrum of bR in the dark-adapted state (orange), the light-adapted state (green), and the post-experiment absorption spectrum (blue). (b) The setup of the grating-based filtering system for the Raman pump pulse. (c) Comparison of ground-state FSRS spectra of cyclohexane with or without the grating-based filtering system. (d) The temporal profile of the Raman pump pulse.

In the FSRS system, to improve the spectral resolution and sensitivity about 100 mW of the Raman pump pulse was introduced into a homemade grating-based spectral filter (see Fig. S1b). Raman pump pulse underwent dispersion through an aluminum reflective grating (1200 grooves/mm, blaze wavelength at 500 nm) and was subsequently focused onto an adjustable slit using an f = 150 mm cylindrical lens. Following the slit, a closely positioned mirror reflected the spatially filtered pulse along the input path. The bandwidth-compressed laser pulse was recollimated by the cylindrical lens and grating and then picked off by a mirror. It is evident that this system effectively enhances the signal-to-noise ratio and significantly improves the frequency-domain resolution of FSRS, as shown in Fig. S1c. The optimal Raman pump pulse, shaped by the grating-based spectral filter, achieved a temporal FWHM of 5.6 ps (see Fig. S1d).

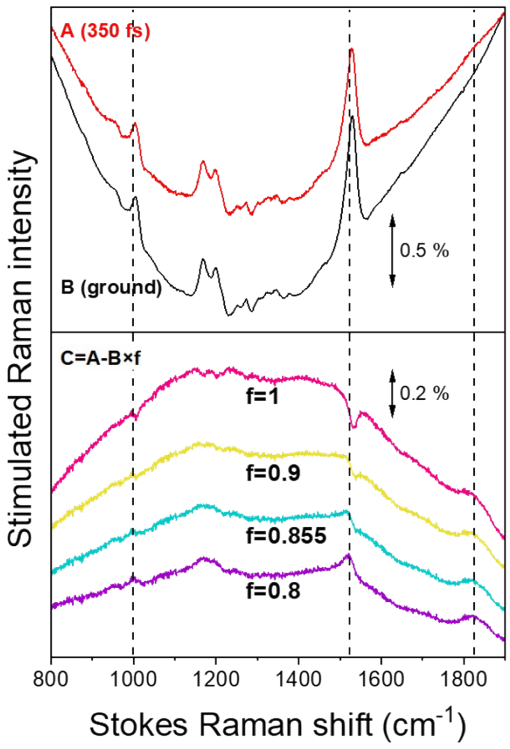

Fig. S2. Data reduction procedure for time-resolved FSRS spectra of bR.

The data reduction procedure for bR is shown in Fig. S2. The top plot shows the ground state data B (black), and the Raman spectrum A (red) at 350 fs after the actinic pump excitation. To isolate the pure excited-state Raman signal C, a scaled fraction of B (B × f, where f is the scaling factor) is subtracted from spectrum A. The scaling factor

f is adjusted until the negative peaks (ground-state bleaching signals) are effectively eliminated. By applying this subtraction method to all delay times, the excited-state Raman spectra shown in Fig. 2 and Fig. S5 are obtained.

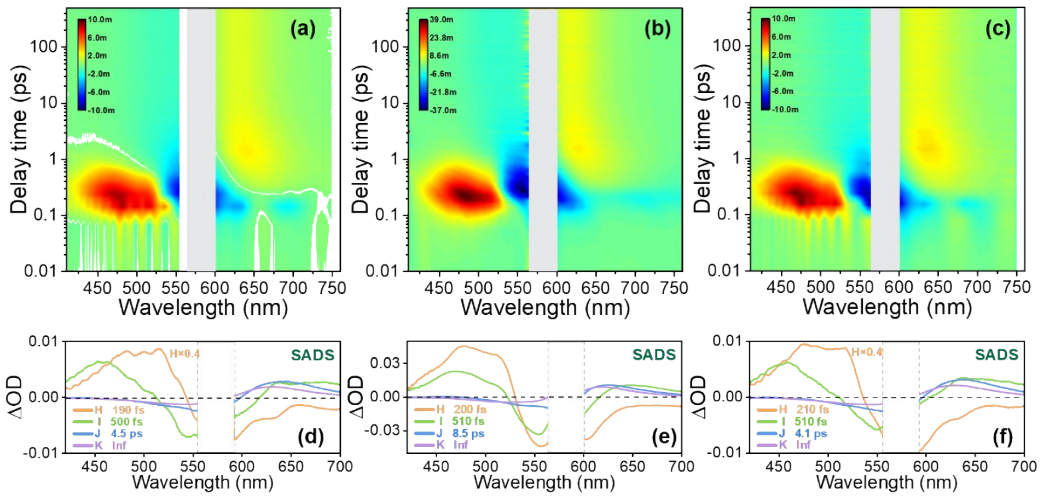

Fig. S3. Two-dimensional contour plots of TA spectra of bR. (a) TA spectra and (d) the species-associated difference spectra (SADS) of bR in light-adopted state under the actinic pump excitation of 50 nJ/ pulse; (b) TA spectra and (e) the SADS of bR in light-adopted state under the actinic pump excitation of 200 nJ/ pulse; The bR sample was kept in the dark overnight after TA measurement of 200 nJ/pulse, and subsequent TA measurement was collected under low excitation power of 50 nJ/ pulse, TA spectra and the SADS for this low-excitation condition are shown in (c) and (f). The gray region indicates the probe wavelength range that is affected by actinic pump scattering.

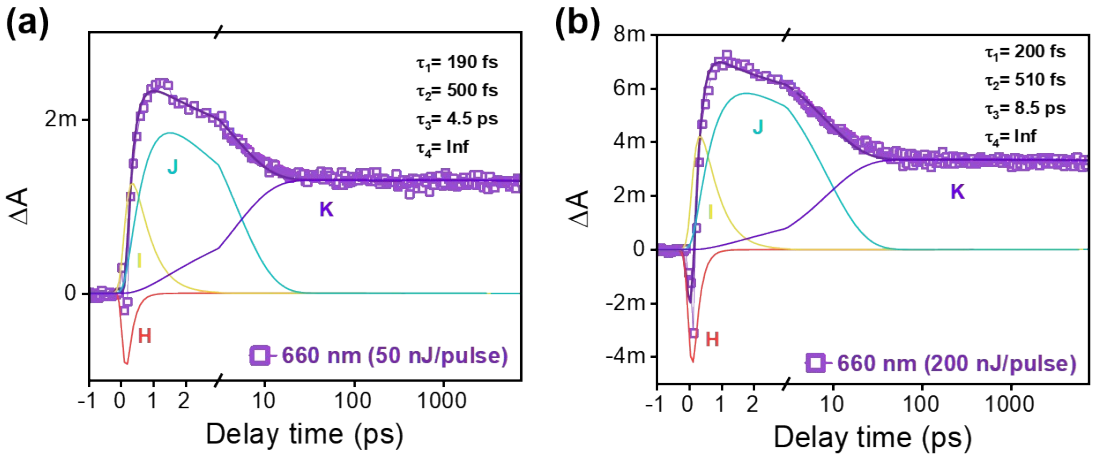

Fig. S4. Transient amplitude of bR in TA spectra probed at 660 nm under the excitation of (a) 50 nJ/pulse and (b) 200 nJ/pulse. Thin solid lines in different colors represent the population and evolution of various intermediate states fitted using SADS, with the lifetimes of intermediates indicated in the figure.

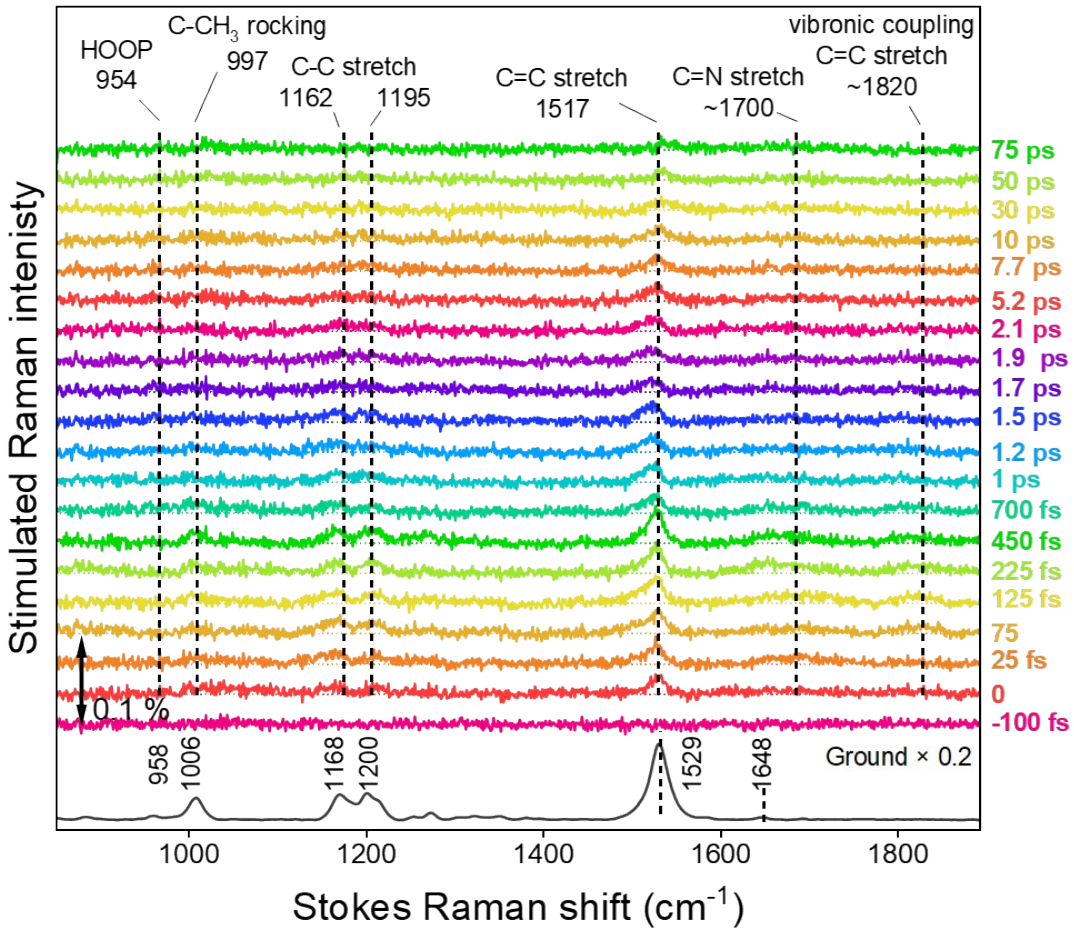

Fig. S5. Excited-state FSRS spectra of all-*trans* retinal in bR following actinic pump excitation of 570 nm, with excitation pulse energy of 50 nJ/pulse.

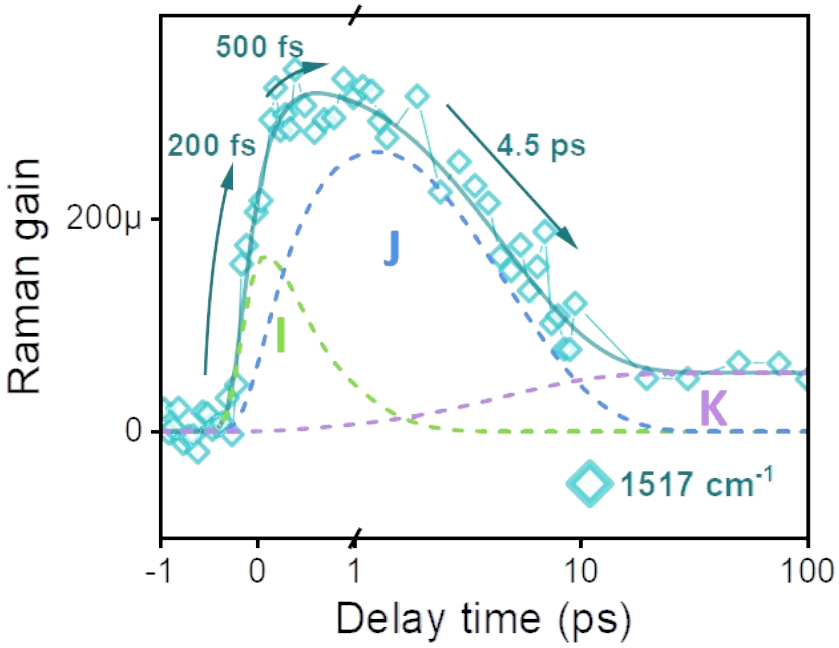

Fig. S6. Transient amplitude of C=C stretching Raman mode at 1517 cm^{-1}obtained with the actinic pump pulse energy of 50 nJ/pulse.

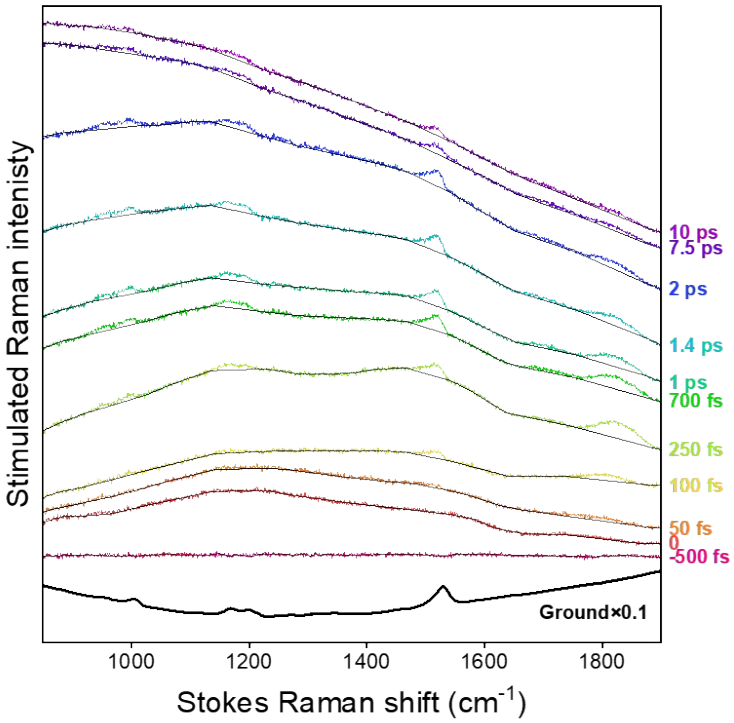

Fig. S7. The raw FSRS experimental data of bR (colored lines) and the corresponding baselines at each delay time (black thin lines). The bottom (black thick line) shows the ground-state bR data without baseline subtraction.

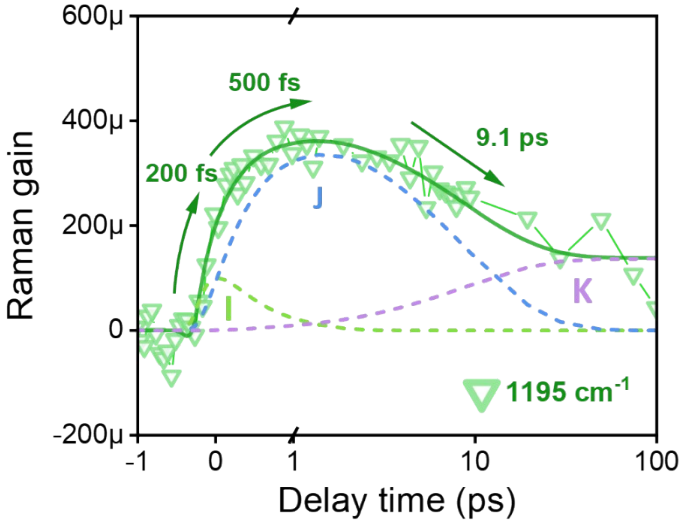

Fig. S8. Transient Raman intensity of the C-C stretching mode at 1195 cm^{-1} with the actinic pump pulse energy of 200 nJ/pulse.